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(54) Title: NOVEL AND IMPROVED TECHNOLOGY FOR PRESERVATION OF ORGANS FOR TRANSPLANTATION

(57) Abstract

A novel method for long-term preservation of organs for transplantation wherein the organ to be transplanted is first perfused with a preservation solution containing essentially pyruvate, inorganic salts providing ions to retain the cell action potential across the membrane and optionally a protein. Then, the organ is perfused with the second preservation solution containing the first solution and alcohol.

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NOVEL AND IMPROVED TECHNOLOGY FOR PRESERVATION OF ORGANS FOR TRANSPLANTATION

Technical Field

This invention concerns a novel and improved process, for long-term preservation of organs for transplantation. The preservation process comprises perfusing the organs such as heart, liver, kidney, pancreas, spleen, brain, embryo, testicles, ovaries, lung or heart-lung complex, or washing organs such as cornea, skin or cartilage, with a first novel physiological preservation solution containing pyruvate, under normal physiological conditions and at a warm temperature to remove blood and other impurities and debris by the increased flow through the organ, bringing the organ to its basal metabolic rate stage with a second preservation solution containing pyruvate and a small percentage of alcohol and preserving the organ with diffusion of gases and nutrients from the media, by submerging and storing the organ in the first preservation solution at low but not freezing temperature for periods longer than 24 hours.

Disclosure of Invention

One aspect of this invention is a novel and improved process for long-term preservation of organs, particularly the heart, liver, kidney, spleen, heart-lung, pancreas, cartillage, skin and cornea for transplantation.

Other aspect of this invention is the preservation of the organ for period 24 hours or longer wherein at that time the organ recovers 90-100% of its original functional activity and around 70% of its intracellular mitochondrial metabolic activity.

Another aspect of this invention is preservation of the organs by sequence of events including a perfusion or washing of the organ, at a warm temperature, with the first preservation solution containing pyruvate for removal of the

blood or other debris from organ's vessels, followed by the perfusion of the organ with the second preservation solution containing pyruvate and small percentage of ethanol in order to vasodilate the blood vessels, prevent edema, and achieve a rapid arrest of metabolic activity, to bring the metabolism to a basal rate stage, and preserving the organ aseptically in the large volume of the first solution for 24 hours or longer at temperatures between 4-10°C.

Still another aspect of this invention is the first novel preservation solution containing an optimal concentrations of ions, sodium chloride, potassium chloride, calcium chloride, sodium bicarbonate, sodium EDTA, magnesium chloride in admixture with sodium pyruvate and a protein.

Yet another aspect of this invention is the second preservation solution comprising the first preservation solution with additionally added ethanol.

Still another aspect of this invention is the prevention of development of metabolic acidosis and edema intracellularly by using the pyruvate to provide energy substrate but to eliminate cellular edema and the production of the acidic metabolite lactate formed during the previously used glucose as an energy supply.

Still yet another aspect of this invention is the decrease or arrest of metabolism achieved by the perfusion of organs with the second preservation solution, which results in a rapid and almost complete metabolic arrest due to the presence of alcohol in the second preservation solution and wherein such metabolic arrest is reversible after periods of storing in the first solution for as long as 24 hours with the recovery of the full functionality of the organ being at that time around 90% of the original activity.

Brief Description of Figures

Figure 1 is a diagrammatic representation of the heart preservation conditions.

Figure 2 is a diagrammatic representation of liver preservation conditions.

Figure 3 depicts ventricular pressure for the heart perfused by cardioplegic solutions in pre-ischemic and post-ischemic hearts.

Figure 4 depicts NMR spectra of hearts pre-ischemic and post-ischemic baseline energy level.

Figure 5 is a diagrammatic representation of kidney preservation apparatus with continuous perfusion.

Figure 6 is a model of the apparatus useful for perfusion of the organs.

Best Mode of Carrying Out the Invention

This invention concerns a novel and improved process for long-term preservation of the organs for transplantation. The preservation process comprises the following steps.

The organ is perfused at a warm temperature with a a first preservation solution containing pyruvate to vasodilate, remove blood, increase flow, and load the cells with an energy supply in the form of a clean substrate, namely pyruvate. Pyruvate prevents edema, ischemia, calcium overload, and acidosis. It also helps preserve the action potential across the cell membrane.

Cannulation of the primary artery or vein incoming to the organ allows for a more complete exchange of gas, substrate, and media during perfusion and storage, thus allowing for usage of pyruvate as an energy source.

Perfusion with a second preservation solution containing both pyruvate and alcohol slows the organ's metabolism, but preserves the energy state of the organ.

Storage of the organ allows the organ to be kept in a closed aseptic container for transportation under conditions which permit diffusion of gases and media during the interim (24 hour or longer) period of transfer while all the time retains the organ in a healthy viable state. Unlike with

other preservation solutions no edema, calcium loading, vasoconstriction or ischemia occurs.

For example, for long-term preservation of the heart, the heart is perfused at a warm temperature with a cardioplegic solution containing pyruvate to vasodilate, remove blood, increase flow, and load the heart cells with an energy supply in the form of a clean substrate, namely pyruvate. A cannulation of the aorta, pulmonary outflow tract, and left ventricular chamber allows for an exchange of gas, substrate, and media during perfusion and storage, thus allowing for usage of pyruvate as an energy source. Perfusion with a second cardioplegic solution containing both pyruvate and alcohol stops the heart from working, vasodilates the vessels allowing for full vascular flow and, continues to load the cells with pyruvate, thus preserving the energy state of the heart. Storage of the heart, via specific techniques, allows the heart to be transported and still preserves the heart's healthy viable state. No edema, calcium loading, vasoconstriction or ischemia occur when this technique is used.

A novel technology for long-term preservation comprises of two novel preservation solutions and the sequential process of using these two solution to achieve the organ preservation for periods of 24 hours or longer after which time the organ recovers 90-100% of its normal functional activity and around 70% of its cellular mitochondrial metabolic activity. Until now no technology or preservation solution has been available to preserve organs for such long periods of time.

This technology is in particular useful for easy transportation of the organ without need to freeze it or without special requirements for unusual refrigerators or freezers. Such transportation may be made in any type or kind of container which allows aseptic conditions, maintenance of temperature of 4-10°C for 24 hours or longer,

as needs be, and a slow influx of oxygen during the preservation period. For extended period of time over 24 hours, the preservation solutions medium contains additional nutrients enabling the basal metabolism to proceed to assure the organ recoverability at the end of preservation period and before a transplantation.

The unique properties of the process are (a) perfusing or washing the organ with a first novel preservation solution (Solution C) containing a pyruvate, protein such as albumin or fetal calf serum and ethylenediaminetetraacetic acid (EDTA), at a warm, preferably room temperature, to remove blood and metabolites from the organ, to load the cells with pyruvate, and to increase the flow of the perfusate through the circulation; (b) serially perfusing the organ with a second novel preservation solution (Solution A) comprising the first solution and a small percentage preferably between 0.01-6%, most preferably around 0.1-4% of ethanol to bring about the reversible decrease in metabolism to the basal metabolic level, prevent edema and retain the integrity of the vascular bed, said perfusion also being performed at a temperature between 4-37°C; and (c) submerging the organ having cannulated primary incoming vein, when appropriate, to allow for diffusion of gases and preservation media, into the large volume of the first solution C for the entire preservation period. The preservation step is maintained at temperature between 2-10°C, preferably for at 4°C for the entire period of the organ preservation.

Unique properties of the preservation solutions are: (a) a presence of pyruvate as a substrate for energy supply demands substituting for generally used glucose which leads to the metabolic acidity and edema causing the damage to the organ function; (b) a presence of protein such as albumin or fetal calf serum useful for cellular recovery and tissue renewal; (c) absence of glucose as an energy source; (d)

absence of phosphate to preserve energy; (e) a presence of EDTA used for removal of harmful ions; and (e) in case of the second preservation solution, the presence of ethanol to affect the reversible decrease in metabolism, prevent edema and retain the integrity of the vascular bed.

The combination of both novel sequential process and novel preservation solutions results in unique technology for preservation of the organs for transplantation for periods six or more time longer than known until now.

Preservation and Cardioplegic Solutions

Successful preservation solution suitable for long-term preservation of the organ must protect the organ against deleterious effects of the long-term induced ischemia which results from the interrupted oxygen supply during the time when the circulation of the donor is stopped before or during the organ removal from the donor and until the organ is connected in the recipient's circulation which assumes and restores the oxygen-blood supply. Since it is well known that for many organs, particularly for the heart, the ischemia extended over 20-30 minutes has often fatal consequences due to and irreversible damage to the organ, it is necessary to design either the preservation solution in such a way that it would prevent ischemia to occur, or to design a conditions which would allow ischemia to occur but would still assure that there is no irreversible damage to the organ and that, after the ischemic period, the organ can resume its normal physiological activity.

In designing the novel cardioplegic solution of this invention, ions are needed to retain the potential difference across the membrane but must be chosen carefully. Some intracellular phosphate (Pi) is needed, too much lowers the energy state. Some Ca^{2+} is needed, too much results in increased work. Some Mg^{2+} is needed, but too much competes with Ca^{2+}. Some KCl is needed but too much leads to vasoconstriction. Bicarbonate is needed to retain the pH in

a physiological state. Sodium chloride is used to balance the osmolarity and retain the action potential across the cell membranes.

As has been discussed above, while the source of energy for the continuous organ activity is necessary even if the organ is in basal metabolic state, it has been found that glucose, which was predominantly used as a source of energy in currently available preservation solutions, causes the acidosis and edema of the tissue which subsequently result in the impairment of the normal function of the tissue. Glucose and fatty acids are more deleterious to the organ during ischemia due to build up of by-products, including accumulation of sugar phosphates. Pyruvate, on the other hand is a beneficial substrate protecting the organs against ischemia, acidosis, edema and a calcium overload. These fundamental observations led to current formulation of a saline solution with pyruvate as the substrate and to the current finding that by substituting glucose in the preservation solution with pyruvate, the tissue acidosis and edema does not occur. Moreover when such solution is combined with alcohol as an agent to arrest the metabolism, a beneficial preservation solution suitable for long-term preservation of the organ results. The discovery described here indicates that using, in certain sequence, the preservation and cardioplegic solutions containing pyruvate, and pyruvate plus ethanol, is able to protect the organ's function for a 24 hour period or longer.

In the absence of work, the organs are able to survive in, and utilize a basal energy state because there is normally an excess concentration of high energy phosphate present in the cells. It appears that when the organ is in a dysfunctional state, the diffusion of oxygen from the oxygenated media is sufficient to maintain the basal energy state of the organ. This occurs at a reduced energy level, but substrate still may be necessary to maintain a viable

basal state. Consequently, when the suitable substrate, such as pyruvate, is provided and when the organ is introduced into the basal energy state by the alcohol, it is able to survive without substantial damage and impairment of its function for at least 24 hours or longer.

Advantages provided by this invention became readily apparent, since the previously known survival of the organs, while timewise differing, due to their functional disruption induced by the removal of the organ from the donor is only somewhere around 2-6 hours, in the case of heart around 2-4 hours, at which time the organ needs to be placed in the recipient's circulation or the irreversible damage or death of the organ occurs.

The organ preservation solution (Solution C, Example 1, page 38) of this invention contains, per liter of deionized or distilled sterile water, 90-120, preferably 17 mM (6.42g) of sodium chloride, 4.0-4.5, preferably 4.3 mM (0.32g) of potassium chloride, 0.5-2.5, preferably 2 mM (0.294g) of calcium chloride, 22-28, preferably 25 mM (2.1g) of sodium bicarbonate, 0-1 mM, preferably 0.5 mM (0.146g) of ethylenediaminetetraacetic acid (EDTA), 1.0-2.0, preferably 1.2 mM (0.144g) of magnesium sulfate or equivalent amount of magnesium chloride, 6-12, preferably 10 mM (1.1g) of sodium pyruvate and 0.05-1%, preferably 0.1% of protein such as serum such as fetal calf serum, serum albumin such as synthetic or natural albumin or any other protein which will provide viscosity similar to the albumin.

Solution A consists essentially of the solution C with added 2-8%, preferably 4% of lower alcohol, such as ethanol and may be used at temperatures from 4°C-37°C. In alternative, alcohol may be substituted with additional 18-26 mM, preferably with 20 mM (1.3g) of potassium chloride which can only be used of temperatures between 2-8°C.

Both solutions may contain additionally 5-20% of fluorocarbon, such as perfluorocarbon obtained from Green

Cross, Japan, preferably in emulsified form.

In another embodiment, the preservation solutions may additionally contain individual essential amino acids or mixtures thereof, or be completely substituted with Eagle or 199 media obtained, for example, from Gibco Laboratories.

Procedure for Organ Preservation

Heart 30 with cannulated aorta, pulmonary artery and with introduced micromanometer catheter in the left ventricle (Fig. 1C) is connected to the perfusion chamber 20 connected to container 40 containing solution C and container 50 containing solution A, both being regulated with valves 42 and 52 respectively (Fig.6). In alternative, liver 30, with cannulated portal vein or any other organ having canulates supply vein (Fig. 2A) is submerged in the perfusion chamber 20 as above. Each container has build-in thermistor to enable to preset and maintain certain temperature. Perfusion by the solution C or A then proceeds. The perfusion with solution C containing pyruvate is designed to bypass the glycolytic pathway and to substitute for glucose as an energy substrate.

Glycolysis in the cells is partially rate-limited by phosphofructokinase, which is inhibited by intracellular calcium $[Ca^{2+}]i$ and hydrogen ions $[H^+]i$ and activated by cAMP. $[H^+]$ and $[Ca^{2+}]i$ are augmented in ischemia. When glucose was used as the only substrate in the preservation solution [NADH]/[NAD], the phosphorylation potential and developed pressure were significantly lower and concentrations of phosphomonoester sugars and hydrogen ions $[H^+]i$ were significantly higher in isolated cardiomyopathic organs as compared to normal organs. Pyruvate on the other hand was shown to lower $[Ca^{2+}]i$. The results published in Basic Res. Cardiol., (1990), suggest that cellular ischemic failure is partially due to calcium and/or hydrogen ion-induced inhibition of glycolysis, which is alleviated by bypassing the glycolytic pathway with pyruvate.

Consequently, the presence of pyruvate in the solution provides unique protection for the organ cells by preventing development of edema, ischemia, calcium overload, acidosis and preserve the cell action membrane potential across the membrane.

The organ is perfused with solution C for 1-20 minutes, preferably for about 10 minutes at a warm temperature between 28°C to 37°C, preferably around 35-36°C. At this temperature, the solution increases flow and loads the cells with an energy supply in the form of a clean substrate pyruvate and vasodilates the organ vessels as opposite to the cold cardioplegia which is known to cause vasoconstriction.

Cannulation of the incoming artery or vein allows the solution to circulate through the whole organ and thus provide exchange by diffusion of gas, substrate and media during perfusion and storage between the solution and the cells. Returning now to the Fig. 6, at the end of perfusion with solution C, the valve 42 is closed and the valve 52 is opened to allow perfusion with solution A containing alcohol. Solution A containing both pyruvate and 0.1-6% of alcohol, preferably 4% of ethanol, causes the arrest of the organs metabolism, vasodilation, and also inhibits the Na^+/Ca^{2+} exchange by preventing the efflux of Ca^{2+}. A combination of low Na^+ and ethanol lowers the influx of Na^+ during the calcium paradox and the efflux of Ca^{2+}. As a result the cell is not depleted of Ca^{2+} during the calcium paradox and not loaded with Ca^{2+} during repletion and the organ is able to recover from the calcium paradox during reperfusion when ethanol is present during the Ca^{2+} - depletion period. To assure a low Ca^{2+} concentration during the Ca^{2+} -depletion period, 0.5 mM EDTA was optionally added to the cardioplegic solutionu.

Perfusion with the second solution (Solution A, Example 1) containing alcohol proceeds for 1-20 minutes,preferably

around 10 minutes at temperatures from 2°C to 37°C. Higher temperatures than 28°C are preferred because they allow for vasodilation but when the circumstances require, the temperature may be lowered to about 4°C which will further slow down metabolism but more importantly will cause certain degree of vasoconstriction. The perfusion with the cold solution containing potassium chloride is preferably done at low temperatures around 4°C.

In alternative, the organ preservation according to the procedure of this invention can be successfully accomplished by perfusion with solution C at warm temperatures (24-37°C) and by subsequent cooling of the solution A to temperatures between 2-10°C, preferably to 4°C. This temperature is also used for the storage period for this procedure wherein the organ cannulated with cannula intact is transferred to the container depicted in Fig. 1B. The only requirements for the container are that it is tightly closed, filled with enough of solution C in such quantity, usually between 3-8 liters, which allows complete submerging of the organ with cannula in the solution, that the aseptic conditions can be preserved including the aseptic supply of gas, preferably oxygen/carbon dioxide 95/5%, and the temperature maintained around 4°C. In alternative, the container may be equipped with the perfusion pump and the organ may be continuously perfused with solution C at temperature between 2-8°C, preferably around 4°C or any other temperature which is needed for its preservation.

Using the procedure of this invention, the organ may be successfully preserved from 24 hours to seven or more days provided that the constant supply of oxygen and essential energy substrate and nutrients is provided. Following the period of preservation but before the organ is transplanted, it may be re-perfused with the solution C to restore its normal physiological functions.

Additional agents, such as drugs, hormones, vitamins

and other pharmaceutically acceptable excipients may be added to solution A or C.

The feasibility of the cardioplegic solutions for long-term heart preservation was studied on animal hearts. Isolated hamster or rat hearts were first perfused with a normal Krebs-Henseleit or Langeidorf medium (Solution B) to demonstrate comparable viability of hearts prior to perfusing and storing for 24 hours in one of three solutions. The three solutions applied to three groups of hearts are described in Example 1 and were: Solution A (second solution) is a cardioplegic solution containing pyruvate as the substrate and 4% alcohol to arrest the heart; Solution B is a standard cardioplegic solution; and Solution C (first solution) is a cardioplegic solution containing physiological saline and pyruvate as the substrate. The testing procedure is a described in Examples 2 and 3. The results are summarized in Tables I and II.

Figure 3 shows a representative drawings of left ventricular pressure for hearts perfused in solutions A, B and C for baseline levels, called preischemia, and 30 minutes reperfusion following 24 hours of storage called postischemia in each solution at 4°C. For solution A, which includes pyruvate and 5% of ethanol, the preischemic and postischemic pressure is almost the same, i.e., the height of peaks is the same and only the rate of the contractions in postischemic heart in solution A is slower. Thus, this solution is able to preserve the pressure and the heart for 24 hours without any apparent impairment in cardiac function. This coincides with results summarized in Table I where the developed pressure and end-diastolic pressures are the same for pre- and post ischemia. Coronary flow and heart rate are somehow slower and the oxygen consumption is about the same. For solution C, containing pyruvate as a substrate results are similar. Although the height of the developed pressure in postischemia is not quite the same as

in preischemia, during the contractions of the heart the pressure returns to 0 levels. Both end-diastolic pressure and coronary flow are the same with heart rate slower and oxygen consumption slightly lower in postischemia. Standard solution B, which has no pyruvate and no alcohol, shows drastical reduction in heart contractility, wherein the postischemic heart contracts only somewhere between 40-70 mm Hg instead of 0-160 as seen in preischemic heart. The results in Table I support these findings. Developed pressure, coronary flow, heart rate and oxygen consumption are all much lower, with end diastolic pressure much higher and the heart looks healthy and normal. On the contrary, the heart stored in standard solution B was edemic and ischemic, probably due to too much of Ca^{++} and lack of available energy substrate, and its function was only about 50%.

Figure 4 A, B and C shows representative 31P-NMR spectra of hearts preischemic (baseline energy level) and postischemic (30 min reperfusion following 24 hours storage) for hearts perfused in solution A (A), solution B (B), and solution C (C).

The upper lower spectra show the preischemic spectra, the upper spectra are postischemic. When the energy level is about the same, the spectra look the same. As seen from spectra A, both upper and lower spectra are about identical, while spectra B, showing the energy level after 24 hours storage in Roe Standard solution (solution B), shows the presence of large amount of inorganic phosphate evidencing intracellular inequilibrium and intracellular acidity. ATP and creatinephosphate (PCr) are grossly diminished when the solution C was used without alcohol, depletion in energy supply is also seen because the heart was not put into the basal metabolic rate state and there was not sufficient supply of energy substrate to support full heart metabolism. The spectra in Figure 4 are confirmed by results summarized

in Table II.

In practice of this invention, the donor's heart, heart-lung complex, kidney, spleen, testicles, ovaries, pancreas, brain or liver are first cannulated as shown in Figures 1 and 2, which depicts a diagrammatic representation of conditions for organ transplantation. The heart aorta and the left ventricle or portal, renal, splenic, pancreatico-duodenal veins, ovaries or spermatic artery are first cannulated, perfused with the warm physiological solution and hemodynamic measurements of baseline metabolic and physiologic levels are made. That state is herein called preischemia. The vascular pressure is measured. The organ is then excised and placed in the perfused working organ apparatus (Fig. 6) and the perfusion with the first preservation solution is started through the cannula connected to container 40 containing Solution C immediately. The perfusion continue for 2 to 60 minutes, preferably for about 5-10 minutes or until all remnants of the blood impurities or debris are washed out from the organ. When the rinsate is clear of blood, the cannular connection is switched to the container 50 with preservation solution A containing 0.01-6%, preferably 0.1-4% of ethanol. The amount of alcohol will depend on the organ. For example, liver and brain perfusate will contain only small amount if at all of alcohol. Both containers are kept at temperature between a room temperature to around 37°C, preferably at the temperature allowing the perfusing solution C and A to have a body temperature, i.e., of about 37°C. The solution A however, may be perfused at temperature 4°C. Nevertheless, a perfusion with colder temperatures as low as 2°C, preferably 4°C is possible and contemplated to the within the scope of this invention.

The controversy connected with the applied temperature of a preservation solution has been discussed above and the detriments connected with freezing of organs or hypothermia

are well documented. Although the most commonly used preservation solution is a cold potassium chloride solution, advantages of warm, oxygenated blood solution were described in J. Thorac. Cardiovasc. Surg., 91:888 (1986). In this invention, perfusing the organ with solutions at warm temperature was found beneficial since it allowed flushing of the veins and organ vessels and prevented them from collapsing. It was also found to be important to remove all blood from the organ since the breakdown of hemoglobin releases, among other things, iron which may have deleterious effects on clotting and inhibition of enzymes.

The perfusion of the organ with the solution A effectively stops the organ work and induces the basal energy state. At this state, in which the organ is in the basal state, as evidenced by the diminished metabolism measured by biochemical techniques known in the art, and having canula/cannulae intact and opened, the organ is transferred to the storage container filled with solution C and maintained at temperature between 2°C-10°C, preferably about 4-15°C. Container can be of any size and shape as long as it contains at least 4-8 liters of solution C so that the cannulated organ including open ends of canulae are submerged at all times in the solution C. One example of such container is shown in Figures 1B and 2B. It is important that the container are that is tightly capped and is equipped to provide slow but continuous influx of oxygen. The container and a solution therein must both be sterile and the influx of oxygen must be done aseptically. The cannulation tubes are provided and are necessary for continuous aeration by diffusion of the cell and tissues while in the storage container. The cannulation tubes are designed so that the physician performing transplantation can connect the cannula to a pressure transducer in order to check pressure in the organ or other parameters before transplanting.

Tightly closed container is connected to a transportable miniature oxygen supply and is transported and transportable anywhere in the whole world by any means of transportation, and as long as it the container not submitted to excessive heat or cold which would damage organ tissue, it can be stored for at least 24 hours or more and the organ can be transplanted without loss of function. Following the properly done transplantation, the isolated organ for transplantation will regain 90-100% of its normal physiological function and around the 70% of its cellular metabolic activity immediately upon connecting the organ to the recipient's circulation.

All organs which can be transplanted are contemplated to be within the scope of this invention. The heart, heart-lung complex, liver, kidney, spleen and pancreas are first cannulated, if possible, in-situ, in the donor's body via any major vein. Then the organ is gently removed under the strictly aseptic conditions accepted in the surgical procedures and the first rinsing of the organ with the warm physiological solution is quickly done. Then the organ, with canula/canulae intact, is quickly removed and transferred to the isolated organ apparatus, as shown in Figure 2, wherein the organ is preferably submerged in the solution with which it is perfused, to prevent drying of the organs surface. In another arrangement, the organ apparatus has enclosed humidified chamber where the organ is hanging or laying on the surface of the supporting structure throughout the perfusion period with solution C (2-20 minutes) and solution A (2-20 minutes), in series. After the basic biochemical and functional tests have been performed, but immediately after the end of the perfusion with the solution A, the organ is gently transferred into storage container and submerged completely including the end of cannula/cannulae in the solution C again and stored for 24 hours or more at low temperature of about 2-10°C,

preferably 4°C.

In alternative, the organ is first perfused with a warm solution C (35-37°C) and when all blood and tissue debris is rinsed out, the perfusate is cooled down and the organ is stored, as above, in the cold solution C at temperature of 4°C. In yet another alternative, the organ is first perfused with solution C at warm temperature and then transferred into cold solution A at about 4°C. Optionally, oxygen carriers such as various fluorocarbons, for example perfluorocarbon in amount from 5-20% can be added to the solution C, A or both. In this instance, the fluorocarbon will be emulsified with for example lecithin, using methods known in the art.

Another alternative contemplated to be within preview of this invention is the replacement of the solution C or solution A with the liquid media, such as Medium 199, available from GIBCO Laboratories, New York, which consist of mixture of essential amino acids, as long as the medium is either mixed with or prepared with the pyruvate in amount from 1-20 mM, preferably in amount 6-12 mM. These medium/pyruvate solutions will be particularly useful for preservation of organs for longer than 24 hours period of time and mainly for preservation of heart, heart-lung, brain and embryo.

In yet another alternative, the solution C or medium/pyruvate combination may be used for continuous perfusion of the organ in the storage chamber. In such practice a portable miniature perfusion pump is connected to the container aseptically and connected to the cannulated organ. The organs are then continuously perfused with medium/pyruvate containing solutions C or A for the whole period of preservation and storage.

Use of alcohol as a preservation solution may have limited use for transplantation unless the alcohol is perfused out of the organ system prior to use. Thus, it is

contemplated to be advantageous to store the organ in the physiological saline containing pyruvate but not alcohol. However, functional recovery in the latter is still better than a standard preservation solution.

Industrial Aplication

This invention is useful for organ transplantation, in particular for the transplantation of organs such as the heart which require, beside suitable preservation solution, also special conditions in order to preserve their physiological function. The invention process is equally suitable for 24 hours preservation of the organ prior to the transplantation as it is suitable for longer than 24 hours preservation of the organ. In such an event, there will be continuous supply of pyruvate and/or other nutrients, whether added to the solutions of this invention or supplied by the medium 199 which contains pyruvate, ions and other agents needed for such extended survival. These extended preservation periods are within the scope of this invention. Various drugs and agents such as hormones, vitamins, nutrients, antibiotics and such others may be added to preservation solutions at any stage of the protective process of this invention as long as the aseptic conditions and safety are maintained.

The following examples are intended to illustrate the invention. They should not be in any way interpreted to limit the scope of this invention.

EXAMPLE 1

Preparation of Cardioplegic Solutions

This example illustrates preparation of cardioplegic solutions for long-term preservation of heart for transfusion.

Solution A

1.07 mM (6.25 g) of sodium chloride, 4.3 mM (320 mg) of potassium chloride, 2 mM (294 mg) of calcium chloride, 25 mM (2.1 g) of sodium bicarbonate, 0.5 mM (146 mg) of sodium

EDTA, 1.2 mM (144 mg) of magnesium sulfate, 10 mM (1.1 g) of sodium pyruvate, 0.1% 1. of fetal calf serum and 4% of ethanol were dissolved at room temperature under constant stirring in 1 liter of deionized distilled sterile water, pH was adjusted to 7.4, and the solution was stored in the refrigerator at 4°C until used for heart perfusion.

Solution B

20 mEq/l of potassium (K^+), 27 mEq/l of sodium (Na^+), 3 mEq/l of magnesium ($Mg\ 2^+$), 47 mEq/l of chloride (Cl-) was dissolved in 1 liter of deionized water. The solution was adjusted to osmolarity 347 and pH 7.6 and stored at 4°C in the refrigerator. The preparation of this solution is described in J. Thorac. Cardiovasc. Surg., 73:366 (1977).

Solution C

1.07 mM (6.25 g) of sodium chloride, 4.3 mM (320 mg) of potassium chloride, 2.0 mM (294 mg) of calcium chloride, 1.2 mM (144 mg) of magnesium sulfate, 25 mM (2.1 g) of sodium bicarbonate, 0.5 mM (146 g) of sodium EDTA and 10 mM (1.1 g) of sodium pyruvate were dissolved, under constant stirring in 1 liter of deionized water, pH was adjusted to 7.4, and the solution was stored at 4°C in the refrigerator. Before use, solutions were oxygenated with mixture of 95/5% of oxygen/carbon dioxide.

EXAMPLE 2

Testing of Cardioplegic Solutions

This example illustrates testing and effect of cardioplegic solutions on the heart preservation.

Animal hearts were obtained from Golden hamsters weighing approximately 140 grams and 18 months of age. The animals were anesthetized with ether. After midline sternotomy the heart was rapidly excised with removal of the pericardium, immediately connected to an aortic perfusion cannula and perfused by a modified Langendorff method described in Am. J. Physiol., 245:H 354 (1983) with a perfusion pressure of 140 cm H_2O. The oxygenated perfusate

was equilibrated with 95% O_2 and 5% CO_2. All hearts were first perfused with a physiological saline [117 mM NaCl, 4.3 mM KCl, 2.0 mM $CaCl_2$, 1.2 mM $MgCl_2$, 0.1 mM K_2HPO_4, 25 mM $NaHCO_3$, 0.5 mM NaEDTA, 15 mM glucose, and 10 units/liter insulin.] After 15 minutes equilibration physiological measurements were made and energy metabolites were studied.

Physiological measurements included oxygen consumption, pO_2, and coronary flow. Oxygen consumption was determined as follows: Arterial samples were aspirated from the aortic chamber and venous samples were drawn from a catheter introduced into the right ventricular outflow tract for oxygen measurements (Corning model 165/2 gas analyzer). PaO_2 was measured and the oxygen content calculated as the product of coronary flow and coronary oxygen extraction. Coronary flow was determined by collecting the effluent of the right ventricle for one minute. A cannula was inserted through the left atrial appendage and into the left ventricular cavity, connected to a Statham P23Db pressure transducer.

Magnetic resonance spectroscopy was performed as follows. 31_p magnetic resonance spectroscopy of the beating isolated perfused heart was obtained on a 5.6 Tesla vertical 76 mm bore magnet as described in Circ. Res., 59:597 (1986). 31_p NMR spectra were obtained without proton decoupling at 97.3 MHz, using a 1180 Nicolet computer, a pulse programmer, and a high resolution 20 mm broad-band probe. Pulse angle was 60°C, recycle time 1.25 sec, and spectra width 4000 Hz. The 512 transients were accumulated during a 10 minute period. The signal to noise ratio was approximately 30:1. To correct for partial saturation, fully relaxed spectra were obtained at 15 second recycle time, and correction factors for phosphocreatine (PCr) and intracellular phosphate (Pi) were determined (3% and 5% respectively). Chemical shifts are referred to the resonance position of PCr. The peaks characteristic peaks of intracellular

phosphate, phosphocreatine and phosphate groups of adenosine triphosphate (ATP) were identified. Intracellular pH was standardized as follows: A standard solution at physiological ionic strength (150 mM KCl, 8 mM ATP, 10 mM PCr, 5 mM Pi, and 9 mM $MgSO_4$) was used at a temperature of 37°C to obtain the chemical shift titration curve of pH dependent Pi to PCr peak difference; this curve was fitted to the Henderson-Hasselback equation. Phosphate peaks were quantitated using manual electronic planimetry and estimated for whole heart detection by comparison to a capillary tube of standard methylene diphosphonic acid fixed inside the NMR tube. High energy phosphate values determined by ^{31}P-NMR were standardized by parallel studies of high pressure liquid chromatography of freeze-clamped tissue Cardiovasc. Res., 20:471 (1986).

After the hearts were perfused with a Krebs-Henseleit solution physiological as well as biochemical measurements performed, the heart was transferred to solution A, solution B or solution C as described in Example 1.

The hearts were perfused for 10 minutes with one of the three solutions. Then, the cannula feeding the aorta was clamped, the cannulae leading to the aortic perfusate and to the pressure transducer were disconnected, and the heart submerged in one of the three respective solutions at 4°C. The details are depicted in Figure 1. It was important that both cannulae were open during the 24 hours of storage for interchange with perfusate and dissolve O_2 in the perfusate. It was also important that no air entered the chamber during this time. After 24 hours of storage at 4°C, the cannulated hearts were again reperfused in a normal Krebs Henseleit medium. For reperfusion the cannula leading to the submerged aorta was clamped and placed in position of the perfusion apparatus. The cannula leading to the left ventricular chamber was again fastened into position.

The stimulator-triggered freeze clamp was attached to

the perfusion apparatus. The pneumatic cylinders were driven at 60 Psi for "smashing" the heart and inducing a drop in temperature of the center of the heart from 37°C to -80°C within 5 msec. The frozen wafer fell from the anvils into liquid nitrogen when the cylinders were retracted using the neutralized extract, high energy phosphates were analyzed by high pressure liquid chromatography as described in detail in IEEE Trans. Biomed. Eng., 29:448 (1982). The nucleotide were separated on a Beckman HPLC with a C-18 reverse phase column. All values were analyzed within a range of linearity.

Data are reported as mean and standard deviation. The unpaired Student t-Test was used for assessing the null hypothesis, and rejected at 95% confidence level.

EXAMPLE 3

Preischemic and Postischemic Cardiac Function

This example illustrates the preischemic and postischemic cardiac function depending on the cardioplegic solution used.

Three groups of isolated hamster hearts were perfused for 10 minutes with a Krebs-Henseleit solution according to procedures described in Example 2 and the base level (preischemia) of developed pressure expressed in mm HG measured in the left ventricle, end of diastolic pressure expressed in mm Hg, coronary flow expressed in ml/minute, heart rate expressed in beat/minute, rate pressure product x 10^3 and oxygen consumption expressed in umoles/g/dry weight/minute was determined. Then, the hearts were perfused with one of the solutions listed in Example 1 as follows: Group I (6 hearts) was perfused with Solution A containing pyruvate and ethanol; Group II (6 hearts) was perfused with Solution B standard cardioplegic solution; and Group III (6 hearts) was perfused with Solution C containing pyruvate. The perfusion lasted about 10 minutes.

The hearts were then submerged for 24 hours in one of

the three solutions described in Example 1. Both canulae were left open to assure the interchange with the perfusate solution and the supply of oxygen from the perfusate via cannulae into the heart. The ischemic period was 24 hours. After 24 hours of storage at 4°C, the cannulated hearts were again reperfused with a normal Krebs-Henseleit medium.

Preischemic, postischemic and control values were then determined and are summarized in Table I.

Preischemic and postischemic cardiac function is shown in Table I for all three groups. Prior to ischemia, among all three groups there was no significant difference in developed pressure, end-diastolic pressure, coronary flow, heart rate and consumption prior to ischemia. In postischemia as compared to preischemia, Group I showed no significant difference in developed pressure, end-diastolic pressure and oxygen consumption, however, there was a small but significant ($p<.05$) decrease in coronary flow, heart rate and the rate-pressure product. Group II, on the other hand, showed a significant ($p<.01$) decrease in all measured hemodynamic parameters, except end-diastolic pressure; in latter there was a significant ($p<.001$) increase in pressure. In Group III there was a significant ($p<.01$) decrease in heart rate, rate-pressure-product, and O_2 consumption, but no significant change in developed pressure, coronary flow and end-diastolic pressure. Between Groups I and III, there was no significant difference in end-diastolic pressure, coronary flow, developed pressure and heart rate, postischemically, however the rate-pressure-product and O_2 consumption were moderately but significantly smaller ($p<.05$) in Group III as compared to Group I. Group II postischemically had a significantly ($p<.01$) lower developed pressure, coronary flow, rate-pressure-product and O_2 consumption as compared to Groups I and III, and a significantly ($p<.001$) higher end-diastolic pressure. Representative tracing of left ventricular

pressure for hearts perfused in Solution A, B, and C are shown in Figure 2 for preischemia (baseline level) and after 30 minutes of reperfusion following the 24 hours of storage (postischemia).

Recovery, based on the rate-pressure-product and oxygen consumption after 30 minutes of reperfusion was 81% and 93%, respectively for Group I, 13% and 32% for Group II, and 70% and 72 % for Group III. Percent of physiological recovery was not related to recovery of ATP.

Retention of the heart in a cold cardioplegic solution was necessary in order to maintain the basal energy state of the heart low. There was an interchange of metabolites and oxygen with the coronaries and ventricular chamber. If the heart was not cannulated, as described, the heart could only survive for a few hours. If the aortic cannula was clamped closed, survival time was 60-90 minutes. Cardiac function was better preserved in hearts perfused with a saline solution cardioplegic 1 containing alcohol and pyruvate as compared to the standard cardioplegic solution.

TABLE I

	I	II	III	IV	V	VI
GROUP I [N=6]						
(Solution A) (Modified Krebs-Henseleit Medium) 5% Alcohol and Pyruvate as the substrate						
Preischemia	155±16	2±1	11±1	218±9	34±2	39±3
Postischemia	148±13	2±1	8±2	184±11	28±2	37±2
GROUP II [N=6]						
(Solution B) Roe's Cardioplegic Solution						
Preischemia	148±12	2±1	11±1	215±8	32±1	38±1
Postischemia	55±11	65±8	5±2	150±12	4±1	12±2
GROUP III [N=6]						
(Solution C) (Modified Krebs Henseleit Medium) Pyruvate as the substrate and no alcohol						
Preischemia	156±9	2±1	10±1	210±9	33±1	35±4
Postischemia	134±13	2±1	10±2	173±12	23±2	25±3

I is developed pressure in mm Hg
II is end diastolic pressure mm Hg
III is coronary flow ml/min
IV is heart rate (BPM)
V is rate pressure product [x10^3]
VI is Oxygen consumption u moles/g/day weight/min

EXAMPLE 4

Preischemic and Postischemic Energy Levels

This example illustrates the preischemic and postischemic energy levels depending in the cardioplegic solution used.

In the same three groups of isolated hamster hearts as described in Example 3, levels of ATP, phosphocreatine, inorganic phosphate [Pi] and intracellular pH $[pH]_2$ were determined. The results are summarized in Table II.

Preischemic and postischemic energy levels are shown in Table II. It was necessary to measure preischemic energy levels by ^{31}P-NMR which is non-invasive so that the hearts would be available for further study. Standardized values obtained from ^{31}P-NMR matched freeze clamped data. The NMR values were normalized and then standardized by parallel freeze clamped data. It was necessary to freeze-clamp in order to obtain the postischemic values since the energy levels were low at this time and difficult to detect by ^{31}P-NMR in the small hamster hearts (approximately 0.6 grams). It was possible, however, to obtain the inorganic phosphate and $[pH]_i$ form the ^{31}P-NMR data. The inorganic phosphate did not rise excessively high since there was no added phosphate in the perfusate. Preischemically there was no significant difference in the energy metabolites among the three groups of animals, nor any difference in [pH]i. Postischemically there was no significant difference in ATP and P_i among the three groups of animals (Table II). On the other hand, PCr was significantly higher in Groups 1 ($p<.001$) and 3 ($p<.01$)

as compared to Group 2. There was no significant difference in $[pH]_i$ between Groups 1 and 3, however the [pH]i was significantly more alkaline in Group 2 ($p<.05$) (Table II). Representative ^{31}P-NMR spectra are shown in Figure 3 for baseline (preischemia) level and reperfusion following 24 hours of storage (postischemia) for hearts perfused and stored in Solution A (A), Solution B (B), and Solution C (C).

The ATP level returned to approximately 40% of control level in all three groups, and in all three groups inorganic phosphate remained approximately 320% over control level after 30 minutes of reperfusion. Phosphocreatine was significantly higher in Groups 1 and 3 as compared to Group 2, related to improved oxygen consumption. Intracellular pH (pH)i, based on ^{31}p-NMR, was physiological in Groups 1 and 3 but alkaline in Group 2. The latter may have been due to leaky membranes. Pyruvate helped to preserve mitochondrial function during depressed oxygen delivery, i.e., during 24 hours storage while 4% alcohol arrested the heart, and along with pyruvate was best for preserving functional recovery.

Alterations in the function of cardiac cellular membranes, which control the electrophysiological and mechanical behavior of cardiac muscle, may contribute to the pathogenesis of abnormal cardiac function. Alcohol affects transmembrane ion fluxes of Na^+, K^+, Ca^{2+}, and Cl^- and inhibits the Na^+/K^+ and the Na^+/Ca^{2+} exchange, thereby reducing energy dependent processes during the basal state. Alcohol further decreases intracellular Na^+ levels, causes dehydration of the myocardial fiber and prevents edema.

As can be seen from the results retention for cardiac function was not related to ATP concentrations, Pi levels, or [pH]i. It is possible that the saline solution containing pyruvate and alcohol helped to maintain membrane integrity by influencing ion distribution. Upon reperfusion the hearts reperfused with Roe's cardioplegic solution had

a slightly alkaline [pH]i. The latter may be due to leaky membranes and inability of the myocardial fibers to maintain the ion gradient. Rapid reversibility of cardiac depression is a desirable feature of a cardioplegic solution. The 4% alcohol caused an immediate arrest and the cardiac depression induced by alcohol was immediately reversible. An immediate arrest may not be crucial for preserving cardiac function since myocardial recovery was nearly as good with the physiological saline having pyruvate as the substrate, as compared to the same solution plus alcohol, and significantly better than a standard cardioplegic solution.

High intracellular calcium $[Ca^{2+}]i$ and low [pH]i, which occurs with ischemia, inhibits glycolysis and fatty acid oxidation, resulting in accumulation of intermediates with no provision for synthesis of high energy phosphates. With reperfusion the PCr levels rose in the hearts of the groups which were provided pyruvate. Commensurate with the rise in PCr there was also an increase in oxygen consumption. Inorganic phosphate did not rise excessively high in any of the three groups; the latter is most likely due to the fact that no inorganic phosphate was added to the media.

The results of this study confirm that a saline solution containing 4% ethanol and 10 mM pyruvate preserves cardiac function over a 24 hour period, and that a saline solution containing 10 mM pyruvate partially preserves cardiac function over a 24 hour period.

TABLE II

	ATP (mM)	PCr (mM)	Pi (mM)	[pH]i
GROUP I [N=6] (Solution A) 5% Alcohol and Pyruvate as the substrate				
PREISCHEMIA	9.8+1.2	11.9+1.1	3.6+0.4	7.05+.02
POSTISCHEMIA	3.8+1.1	8.6+1.4	10.4+0.9	6.95+.03
GROUP II [N=6] (Solution B) Roe's Cardioplegic Solution				
PREISCHEMIA	10.1+0.8	12.2+2.1	3.3+1.0	7.01+.02
POSTISCHEMIA	4.2+0.9	3.2+1.2	9.6+1.4	7.22+.07
GROUP III [N=6] (Solution C) Pyruvate as the substrate and no alcohol				
PREISCHEMIA	9.7+1.2	11.8+2.1	3.8+0.8	7.02+.03
POSTISCHEMIA	3.6+2.1	6.2+1.4	11.8+1.4	6.97+.06

EXAMPLE 5

Isolated Pig Heart Preserved for 24 Hours

This example illustrates the utility of the present invention for the preservation of the isolated heart of the higher mammal species.

Pig heart was precannulated as described before and removed from the pig's chest according to procedures described in Example 2. Preischemic measurements were done to determine levels of the same parameters for cardiac function and energy level as in Example 3. Then the heart was perfused for 15 minutes with cardioplegic Solution C and 10 minutes with cardioplegic Solution A. Both perfusions were done at 37° C. The isolated heart was then submerged in a 2 gallon container tightly closed and filled with the Solution C for 24 hours at a temperature 4° C. The heart, including cannulae was completely submerged in the Solution C which was continuously gently oxygenated with a mixture of 95% of O_2 and 5% of CO_2. All conditions were kept aseptic and the apparatus was sterilized beforehand. The heart was attached gently to the wall of the container wall so that no mechanical damage occurred during the simulated

transportation from the one room to another. After 24 hours, the heart with both cannulae was removed from the container, reattached to the isolated heart apparatus and reperfused with the solution C again. The vital signs were followed.

At that time, more than 90 % of the normal cardiac function of the myocardium and more than 70% of the mitochondrial activity was restored, measured by pressure, heart beat, coronary flow and ECG and ^{31}P-NMR.

EXAMPLE 6

Preservation of Isolated Liver for Transplantation

This example illustrates the long-term preservation of liver for transplantation.

A. The portal vein and the bile duct of the rat liver are cannulated, removed, transferred to perfusion chamber and perfused 10 minutes with the physiologic solution at 37°C. The perfusion is then switched to the solution A described in Example 1 and perfused for 10 minutes at 37°C temperature for another 10 minutes. Samples and the level of transaminases are determined to show the degree of liver function. After 10 minutes, the perfusion is disconnected and the liver are submitted to ^{31}P-NMR analysis as described in Example 2.

Liver is then transferred to the storage container filled with solution A and maintained at 4°C as shown in Figure 1B. Care is taken that the portal cannula is completely submerged.

Prior to the organ transfer, the container is preoxygenated with filtered mixture of oxygen and carbon dioxide 95/5% from the gas tank source kept outside of container. The container and the solution are kept aseptic at any time.

The liver are gently submerged in the solution A and stored for 24 hours. After 24 hours, liver is removed and the biochemical, enzymatic tests are performed and

physiological function is determined by using ^{31}P-NMR.

B. The liver are treated with solution A and 2 in the same way as described in A.

Storage container connected with the attached portable perfusion pump is prepared as in A and the liver is connected via the portal cannula to the perfusion pump. Perfusion is maintained at steady rate of 4 ml per minute. The production of bile is followed as one of the sign of normal functional capability. The perfusion is done at 4°C with the solution A.

C. The liver are treated as in A but storage solution A contains 10% of emulsified perfluorocarbon.

D. The liver are treated as in B but storage solution A contains 10% of emulsified perfluorocarbon.

E. The liver are treated as in A by it is perfused first with solution A at 37°C for 10 minutes, then the temperature is dropped to 4°C and then the liver is transferred to storage container and stored at 4°C in solution A for 24 hours.

Under all the above conditions, the liver retains its functional and anatomical integrity as evidenced by the biochemical tests and by ^{31}P-NMR for more then 7 days.

EXAMPLE 7

Preservation of Isolated Kidney for Transplantation

This example illustrates the long-term preservation of kidney for transplantation.

A. The renal vein of the rat kidney is cannulated, removed, transferred to perfusion chamber and perfused 10 minutes with the physiologic solution at 37°C. The perfusion is then switched to the solution A described in Example 1 and perfused for 10 minutes at 37°C temperature for another 10 minutes. After 10 minutes, the perfusion is disconnected and the kidney is submitted to ^{31}P-NMR analysis as described in Example 2 for determination of functionality.

Kidney is then transferred to the storage container filled with solution A and maintained at 4°C as shown in Figure 1B. Care is taken that the renal cannula is completely submerged.

Prior to the organ transfer the container is preoxygenated with filtered mixture of oxygen and carbon dioxide 95/5% from the gas tank source kept outside of container. The container and the solution are kept aseptic at any time.

The kidney is gently submerged in the solution A and stored for 24 hours. After 24 hours, kidney is removed and the biochemical, enzymatic, and physiological functions are determined by using ^{31}P-NMR and other tests known in the art.

B. The kidney are treated in the same way as described in A.

Storage container connected with the attached portable perfusion pump is prepared as in A and the kidney connected via the renal cannula to the perfusion pump. Perfusion is maintained at steady rate of 3 ml per minute. The perfusion is done at 4°C with the solution A.

C. The kidney is treated as in A but storage solution A contains 10% of emulsified perfluorocarbon.

D. The kidney is treated as in B but storage solution A contains 10% of emulsified perfluorocarbon.

E. The kidney is treated as in A by perfusing it first with solution A at 37°C for 10 minutes, then the temperature is dropped to 4°C and the kidney is transferred to storage container and stored at 4°C in solution A for 24 hours.

Under these conditions, the kidney retain its functional and anatomical integrity as evidenced by the biochemical tests and by ^{31}P-NMR for about 5 days or longer.

EXAMPLE 8

Preservation of Isolated Spleen for Transplantation

This example illustrates the long-term preservation of spleen for transplantation.

A. The splenic artery of the rat spleen are cannulated, and the spleen is removed, transferred to perfusion chamber and perfused 10 minutes with the physiologic solution at 37°C. The perfusion is then switched to the solution A described in Example 1 and perfused for 10 minutes at 37°C temperature for another 10 minutes. After 10 minutes, the perfusion is disconnected and the spleen is submitted to ^{31}P-NMR analysis as described in Example 2.

Spleen is then transferred to the storage container filled with solution A and maintained at 4°C as shown in Figure 1B. Care is taken that the splenic cannula is completely submerged.

Prior to the organ transfer the container is preoxygenated with filtered mixture of oxygen and carbon dioxide 95/5% from the gas tank source kept outside of container. The container and the solution are kept aseptic at any time.

The spleen is gently submerged in the solution A and stored for 24 hours. After 24 hours, spleen is removed and the biochemical and physiological functions are determined by using ^{31}P-NMR as described before.

B. The spleen is treated in the same way as described in A.

Storage contained connected with the attached portable perfusion pump is prepared as in A and the spleen is connected via the portal cannula to the perfusion pump. Perfusion is maintained at steady rate of 1 ml per minute. The perfusion is done at 4°C with the solution A.

C. The spleen is treated as in A but storage solution A contains 10% of emulsified perfluorocarbon.

D. The spleen is treated as in B but storage solution A contains 10% of emulsified perfluorocarbon.

E. The spleen is treated as in A by perfusing it first with solution A at 37°C for 10 minutes, then the temperature is dropped to 4°C and the spleen is transferred to storage

container and stored at 4°C in solution A for 24 hours.

Under these conditions, the spleen retains its functional and anatomical integrity as evidenced by the biochemical tests and by ^{31}P-NMR for more than 10 days.

EXAMPLE 9

Preservation of Isolated Brain for Transplantation

This example illustrates the long-term preservation of brain for transplantation.

A. The left carotid artery is cannulated, and the brain is carefully removed from the rat's skull with care being taken that no injury to the brain occurs and that the cannula is at all time connected to the perfusion pump and the brain is perfused. The brain is first perfused 10 minutes with the physiologic solution at 37°C. The perfusion is then switched to the solution A described in Example 1 and perfused for 10 minutes at 37°C temperature for another 10 minutes. After 10 minutes, the perfusion is switched back to perfusion with solution A. The brain is submitted to ^{31}P-NMR analysis as described in Example 2.

Isolated brain with constant perfusion going on is then transferred extremely carefully to the storage container having a soft support on which the brain rests and which is filled with solution A and maintained at 4°C as shown in Figure 1B. Care is taken that the carotid artery cannula is completely submerged and immediately connected to perfusion pump.

Prior to the organ transfer the container is preoxygenated with filtered mixture of oxygen and carbon dioxide 95/5% from the gas tank source kept outside of container. The container and the solution are kept aseptic at any time.

The brain are gently submerged in the solution A and stored for 24 hours. After 24 hours, brain is removed and the biochemical under constant perfusion and physiological functions are determined by using ^{31}P-NMR.

B. The brain is treated in the same way as described in A.

Storage contained connected with the attached portable perfusion pump is prepared as in A and the brain is connected via the portal cannula to the perfusion pump. Perfusion is maintained at steady rate of 4 ml per minutes. The perfusion is done at 4°C with the solution A containing medium 199 with essential amino acids.

C. The brain is treated as in A but storage solution A contains 10% of emulsified perfluorocarbon.

D. The brain is treated as in B but storage solution A contains 10% of emulsified perfluorocarbon.

E. The brain is treated as in A by perfusing it first with solution A at 37°C for 10 minutes, then the temperature is dropped to 4°C and then transferred to storage container and stored at 4°C in solution A for 24 hours.

Under these conditions, the brain retain its functional and anatomical integrity as evidenced by the biochemical tests and by ^{31}P-NMR for 24 hours.

EXAMPLE 10

Preservation of Isolated Cartilage, Cornea and Skin for Transplantation

Skin, cartilage or cornea are aseptically removed from the donor and dropped into the perfusion chamber with the perfusion chamber with warm (37°C) circulating solution A. After 10 minutes, when the organs are thoroughly washed from all remnants of the blood and tissue debris, they are gently removed and dropped into container solution A at 4°C oxygenated with mixture of oxygen and carbon dioxide, as described in Example 6 and stored until transplanted.

These organs stored in the solution A are fully functional after 2 months of storage.

CLAIMS:

1. A cardioplegic preservation solution suitable for long-term preservation of the heart for transplantation, comprising pyruvate, inorganic salts providing ions to retain the heart cell action potential across the membrane, and a protein selected from the group consisting of albumin, fetal calf serum, or other protein providing viscosity similar to albumin.

2. The solution of Claim 1 wherein the inorganic salts providing the ions are sodium chloride, potassium chloride, calcium chloride, sodium bicarbonate, sodium ethylenediaminetetraacetic acid, magnesium salt.

3. The solution of Claim 2 wherein the protein is fetal calf serum, synthetic or natural albumin, and magnesium salt is magnesium chloride or magnesium sulfate.

4. The solution of Claim 3 comprising 90-120 mM of sodium chloride, 4-4.5 mM of potassium chloride, 0.5-2.5 mM of calcium chloride, 22-28 mM of sodium bicarbonate, 0.0.5 mM of sodium ethylenediaminetetraacetic acid, 0.8-2 mM of magnesium sulfate or magnesium chloride, 6-15 mM of pyruvate and 0.01-1% of fetal calf serum or albumin.

5. The solution of Claim 4 comprising 110 mM of sodium chloride, 4.3 mM of potassium chloride, 2 mM calcium chloride, 25 mM sodium bicarbonate, 0.5 mM of sodium ethylenediaminetetraaacetic acid, 1.2 mM of magnesium sulfate, 10 mM of sodium pyruvate and 0.1% of fetal calf serum.

6. A cardioplegic preservation solution suitable for long-term preservation of heart for transplantation comprising pyruvate, inorganic salts providing ions to retain the heart cell action potential across the membrane, a protein selected from the group consisting of albumin and fetal calf serum or other protein providing viscosity similar to albumin, and ethanol.

7. The solution of Claim 6 wherein the inorganic salts providing the ions are sodium chloride, potassium chloride, calcium chloride, sodium bicarbonate, sodium ethylenediaminetetraacetic acid magnesium salt and a protein.

8. The solution of Claim 7 wherein the protein is fetal calf serum, synthetic or natural albumin and magnesium salt is magnesium chloride or magnesium sulfate.

9. The solution of Claim 8 comprising 90-120 mM of sodium chloride, 4-4.5 mM of potassium chloride, 0.5-2.5 mM of calcium chloride, 22-28 mM of sodium bicarbonate, 0.0.5 mM of sodium ethylenediaminetetraacetic acid, 0.8-2 mM of magnesium sulfate or magnesium chloride, 6-15 mM of pyruvate 2-8% of ethanol and 0.01-1% of fetal calf serum or albumin.

10. The solution of Claim 9 comprising 110 mM of sodium chloride, 4.3 mM of potassium chloride, 2 mM calcium chloride, 25 mM sodium bicarbonate, 0.5 mM of sodium ethylenediaminetetraacetic acid, 1.2 mM of magnesium sulfate, 10 mM of sodium pyruvate, 4% of ethanol and 0.1% of fetal calf serum.

11. A method for preservation of the heart for transplantation comprising perfusion of the heart with a cardioplegic solution comprising 90-120 mM of sodium chloride, 4-4.5 mM of potassium chloride, 0.5-2.5 mM of calcium chloride, 22-28 mM of sodium bicarbonate, 0.0.5 mM of sodium ethylenediaminetetraacetic acid, 0.8-2 mM of magnesium sulfate or magnesium chloride, 6-15 mM of pyruvate, and 0.01-1% of fetal calf serum or albumin.

12. A method for preservation of the heart for transplantation comprising of first perfusion of the heart with cardioplegic solution containing pyruvate at 37°C, followed with a perfusion of the heart with a cardioplegic solution containing pyruvate and ethanol at temperature from 4-37°C and storing the heart in a cardioplegic solution containing pyruvate at temperature between 2-10°C.

13. An organ preservation solution suitable for long-term preservation of liver, kidney, spleen, heart-lung, pancreas, cartilage, skin and cornea for transplantation, comprising pyruvate, inorganic salts providing ions to retain the cell action potential across the membrane and a protein selected from the group consisting of albumin and fetal calf serum.

14. The solution of Claim 13 wherein the inorganic salts providing the ions are sodium chloride, potassium chloride, calcium chloride, sodium bicarbonate, sodium ethylenediaminetetraacetic acid, and magnesium salt.

15. The solution of Claim 14 wherein the protein is fetal calf serum, synthetic or natural albumin, and magnesium salt is magnesium chloride or magnesium sulfate.

16. The solution of Claim 15 comprising 90-120 mM of sodium chloride, 4-4.5 mM of potassium chloride, 0.5-2.5 mM of calcium chloride, 22-28 mM of sodium bicarbonate, 0.0.5 mM of sodium ethylenediaminetetraacetic acid, 0.8-2 mM of magnesium sulfate or magnesium chloride, 6-15 mM of pyruvate and 0.01-1% of fetal calf serum or albumin.

17. The solution of Claim 16 comprising 110 mM of sodium chloride, 4.3 mM of potassium chloride, 2 mM calcium chloride, 25 mM sodium bicarbonate, 0.5 mM of sodium ethylenediaminetetraacetic acid, 1.2 mM of magnesium sulfate, 10 mM of sodium pyruvate and 0.1% of fetal calf serum.

18. An organ preservation solution suitable for long-term preservation of liver, kidney, spleen, heart-lung, pancreas, cartilage, skin or cornea for transplantation comprising pyruvate, ethanol, inorganic salts providing ions to retain the cell action potential across the membrane, and a protein selected from the group consisting of albumin and fetal calf serum.

19. The solution of Claim 18 wherein the inorganic salts providing the ions are sodium chloride, potassium chloride, calcium chloride, sodium bicarbonate, sodium ethylenediaminetetraacetic acid, and magnesium salt.

20. The solution of Claim 19 wherein the protein is fetal calf serum, synthetic or natural albumin and magnesium salt is magnesium chloride or magnesium sulfate.

21. The solution of Claim 20 comprising 90-120 mM of sodium chloride, 4-4.5 mM of potassium chloride, 0.5-2.5 mM of calcium chloride, 22-28 mM of sodium bicarbonate, 0.0.5 mM of sodium ethylenediaminetetraacetic acid, 0.8-2 mM of magnesium sulfate or magnesium chloride, 6-15 mM of pyruvate 0.1-6% of ethanol and 0.01-1% of fetal calf serum or albumin.

22. A method for preservation of the liver, kidney, spleen, heart-lung, pancreas, cartilage, skin or cornea for transplantation by perfusion of the liver, kidney, spleen, heart-lung, pancreas, cartilage, skin or cornea with a preservation solution comprising 90-120 mM of sodium chloride, 4-4.5 mM of potassium chloride, 0.5-2.5 mM of calcium chloride, 22-28 mM of sodium bicarbonate, 0.0.5 mM of sodium ethylenediaminetetraacetic acid, 0.8-2 mM of magnesium sulfate or magnesium chloride, 6-15 mM of pyruvate, and 0.01-1% of fetal calf serum or albumin.

23. A method for preservation of the liver, kidney, spleen, heart-lung, pancreas, cartilage, skin or cornea for transplantation by perfusion of the liver, kidney, spleen, heart-lung, pancreas, cartilage, skin and cornea with a preservation solution comprising 90-120 mM of sodium chloride, 4-4.5 mM of potassium chloride, 0.5-2.5 mM of calcium chloride, 22-28 mM of sodium bicarbonate, 0.0.5 mM of sodium ethylenediaminetetraacetic acid, 0.8-2 mM of magnesium sulfate or magnesium chloride, 6-15 mM of pyruvate, 0.1-6% of ethanol, and 0.01-1% of fetal calf serum or albumin.

24. A method for preservation of the liver, kidney, spleen, heart-lung, pancreas, cartilage, skin or cornea for transplantation comprising a perfusion of the liver, kidney, spleen, heart-lung, pancreas, cartilage, skin or cornea with a first preservation solution consisting essentially of pyruvate and inorganic salts providing ions to retain the cell action potential across the cell membrane at 37°C temperature, followed with a perfusion of the liver, kidney, spleen, heart-lung, pancreas, cartilage, skin and cornea with a second solution consisting essentially of pyruvate, ethanol inorganic salts providing ions to retain the cell action potential across the cell membrane at temperature from 4-37°C and storing the liver, kidney, spleen, heart-lung, pancreas, cartilage, skin or cornea in the first solution at temperature between 2-10°C.

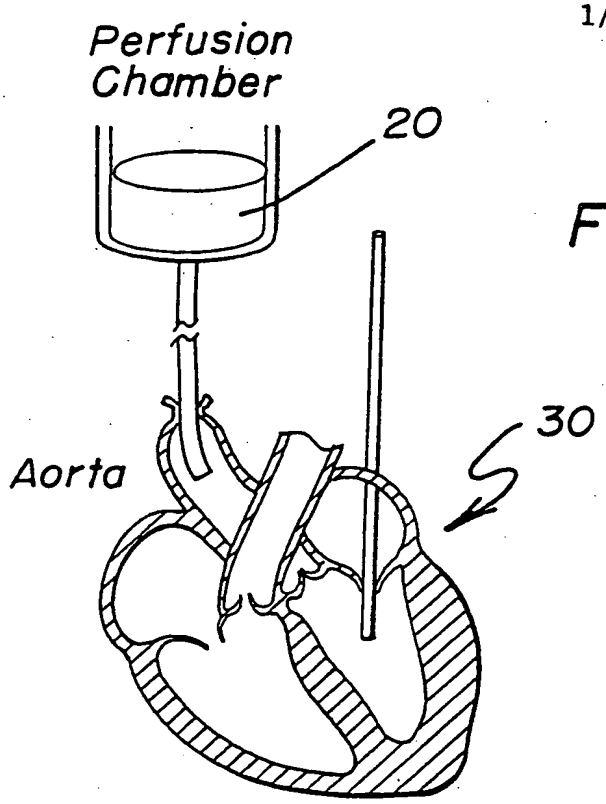
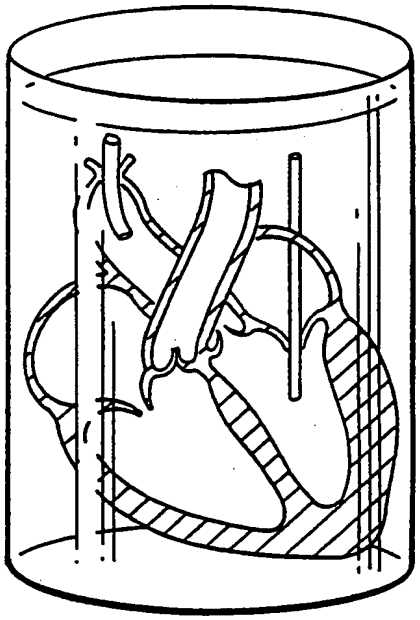


FIGURE IA

FIGURE IB



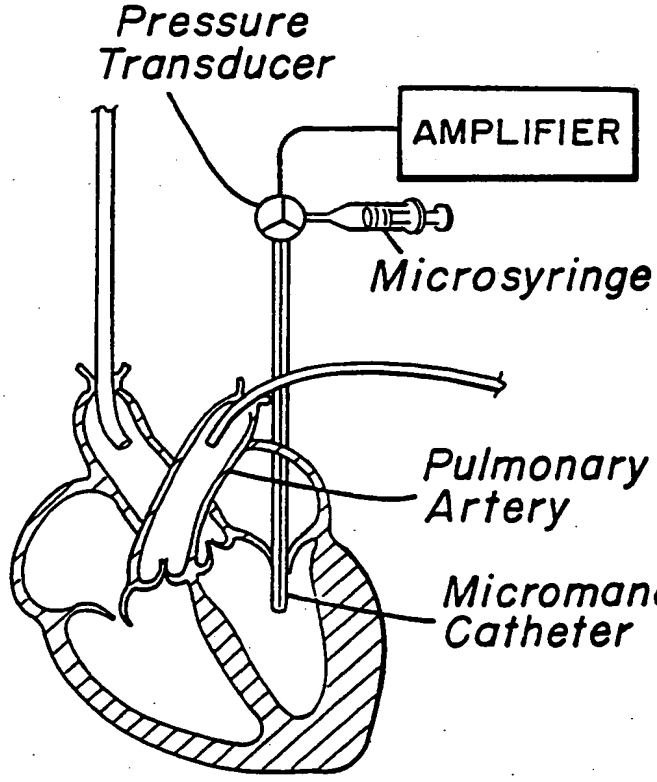


FIGURE IC

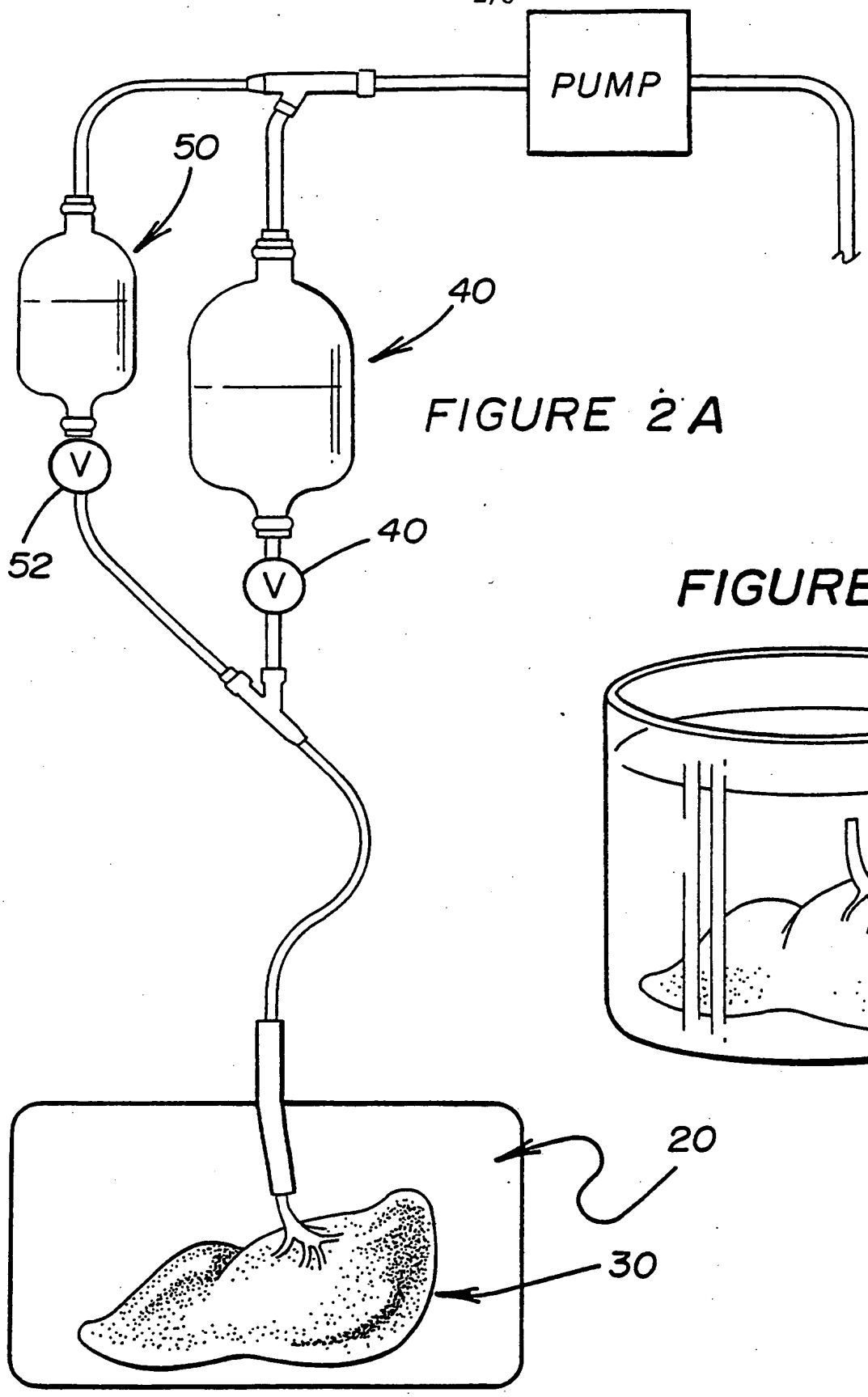


FIGURE 2A

FIGURE 2B

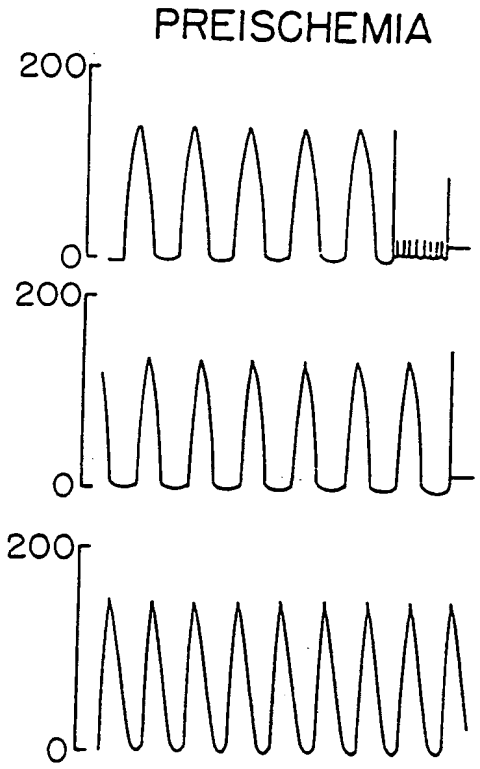


FIGURE 3A

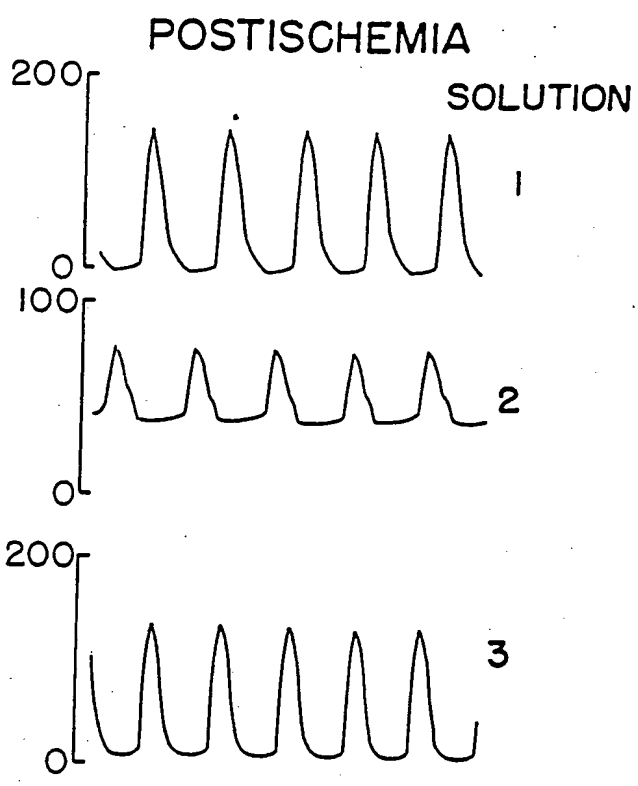


FIGURE 3B

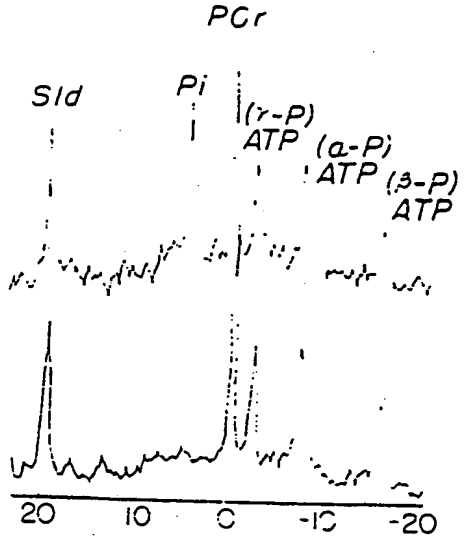


FIGURE 4A

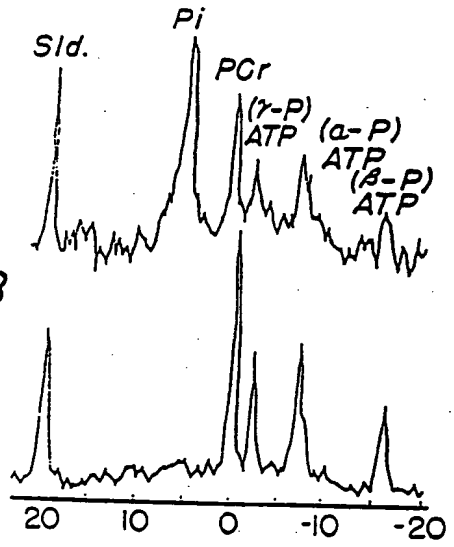


FIGURE 4B

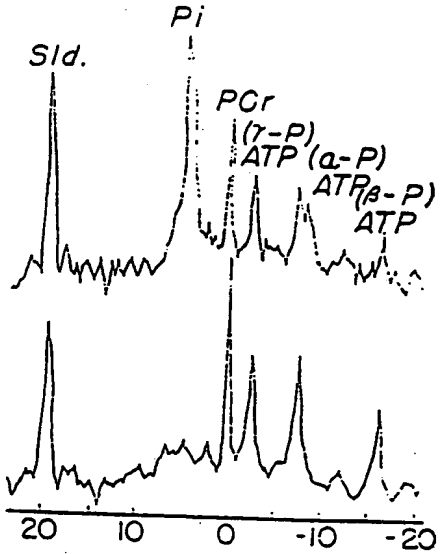


FIGURE 4C

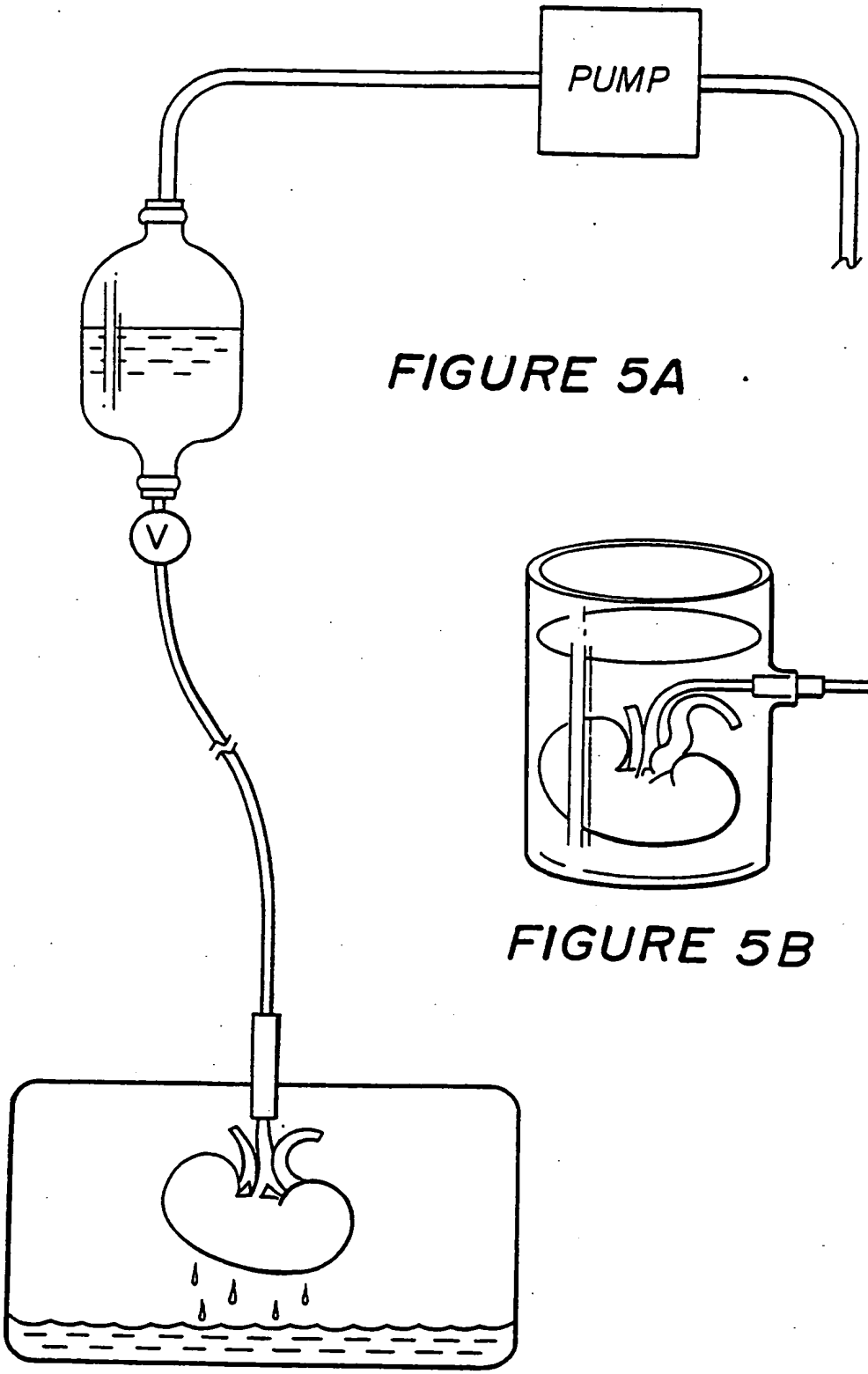


FIGURE 5A

FIGURE 5B

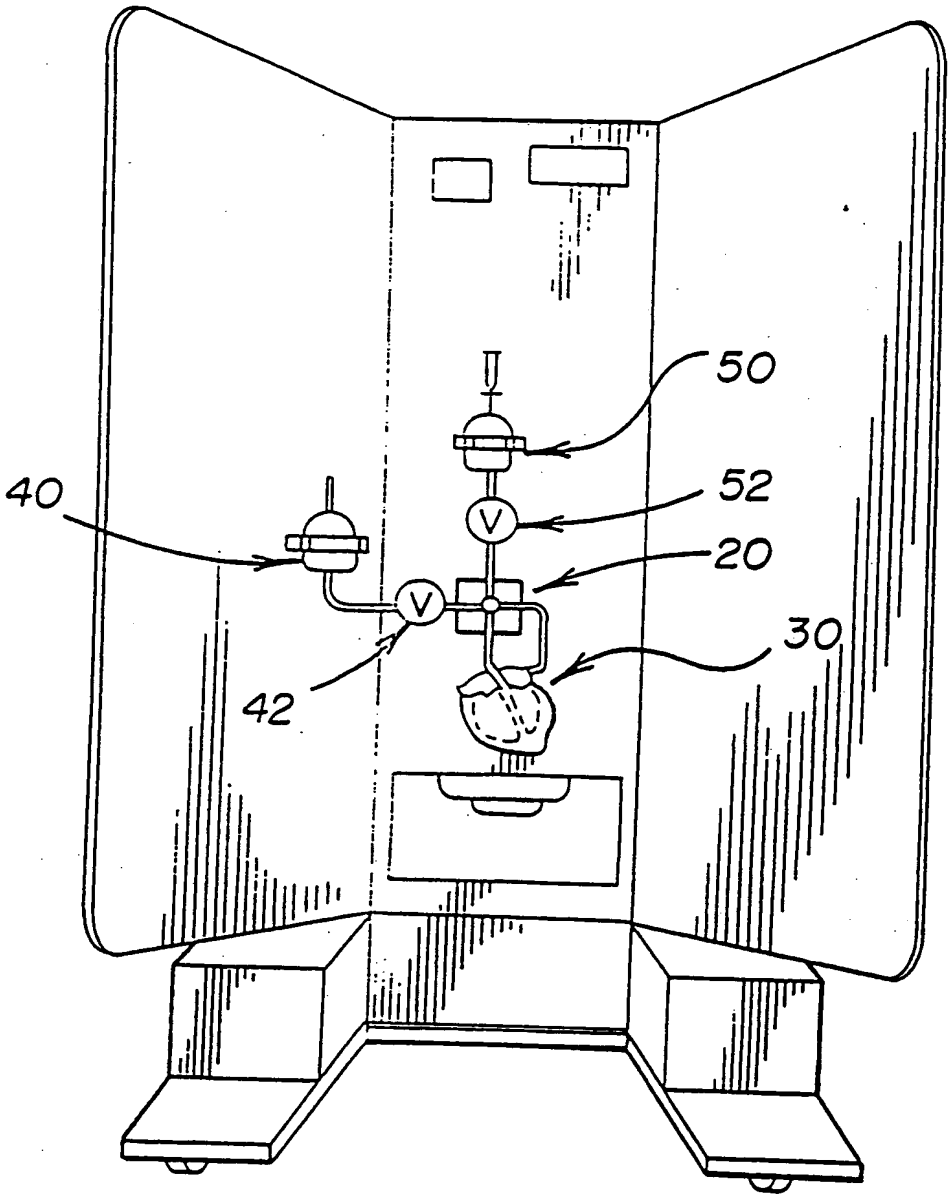


FIGURE 6

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US90/07569

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A01N 1/02
U.S. CL.: 435/1

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/1

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	US, A, 4,663,289 (**VEECH**) 05 May 1987, See col 5, Table II.	1-6, 13-15
P,X	US, A, 4,959,319 (**SKELNIK ET AL.**) 25 September 1990 See col. 6, lines 35+.	1-6, 13-15

* Special categories of cited documents. [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

28 March 1991

Date of Mailing of this International Search Report

22 APR 1991

International Searching Authority

ISA/US

Signature of Authorized Officer

Sam Rosen

Sam Rosen (vsh)

Form PCT/ISA/210 (second sheet) (Rev. 11 87)